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Microbiological quality of some fresh wild edible mushrooms

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ABSTRACT

Fresh mushrooms are an ideal medium for microbial growth because they have high moisture content and a neutral pH. Mushrooms are hand-harvested and exposed to ambient conditions until commercialised in many cases. These characteristics limit their post-harvest shelf life to a few days. Unlike other fresh products, the studies on the microbiological profile of fresh mushrooms are limited. In public, *Lactarius* species are known as "çıntar, melki, kanlıca, termite, menne specifically used in Türkiye. These mushrooms usually grow naturally during the autumn. They are collected by locals and sold in public markets and along the roadside. In this study, the microbiological quality of wild edible *Lactarius* species sold in local marketplaces in the province of Muğla was determined. The results are in the ranges 6.10-8.83 log CFU/g for total mesophilic aerobic bacteria, <1.00-5.57 log CFU/g for lactic acid bacteria, 3.10-7.76 log CFU/g for total yeast, <1.00-3.93 log CFU/g for total mould, <1.00-4.74 log CFU/g for *Staphylococcus aureus*, <0.30-5.07 log MPN/g for total coliform and <0.30-5.07 log MPN/g for faecal coliform. The samples' pH and titratable acidity values were 6.82-7.54 and 0.005-0.020%, respectively.

Keywords: Çıntar, *Lactarius,* Wild mushroom, Microbiological quality, Food safety

Introduction

Macrofungi are used as food as well as medicine in many countries. Macrofungi can be an essential food source for the rapidly increasing world population (Dülger et al., 1999). Besides having a role as food, macrofungi are also a source of income. They are collected, consumed and sold in over 85 countries (Boa, 2004).

Macrofungi can be divided into three groups: edible, inedible and poisonous. Edible mushrooms are collected from nature by people. People have learned which mushrooms are edible from their ancestors or by asking each other (Karasüleymanoğlu, 2014).

Türkiye has a rich edible mushroom flora due to its phytogeographic location. The richness of the mushrooms, which usually appear in spring and autumn, is undoubtedly due to the suitability of ecological conditions. Although Türkiye is rich in edible mushroom species, our people do not know enough about them. It is reported that only 3-5 mushroom species are recognised and consumed as food by the local people (Karasüleymanoğlu, 2014; Altuntaş et al., 2016; Çınar Yılmaz and Işıloğlu, 2016). Some mushroom species, which are well known and eaten in some regions of our country, are not known in other regions and are sometimes considered poisonous. Many types of mushrooms are exported from Türkiye as fresh, chilled, frozen, in brine or dried. *Lactarius deliciosus* (L.) Gray, *Craterellus cornucopioides* (L.) Pers, *Amanita caesarea* (Scop.) Pers, *Hydnum repandum* L., and *Cantharellus cibarius* Fr. are some exported species (Erdoğan et al., 2017). *Lactarius* species are known as çıntar, melki, kanlıca, and termit consumed in many regions of Türkiye (Adanacıoglu et al., 2017). They have a very high market share. They are known as "Çıntar" in the Muğla region and are consumed by the locals. These mushrooms usually grow naturally in autumn and are common in pine and oak forests. Local people are collecting these mushrooms and sell them in public markets in Türkiye (Allı et al., 2006; Pekşen et al., 2007; Yılmaz and Bengü, 2018; Allı, 2022).

Mushrooms are distributed worldwide, and their consumption has increased in recent years due to their nutritional value and organoleptic attributes (Ergönül et al., 2018; Avcı and Avci, 2019). Mushrooms are characterised as valuable nutrients due to their content of fatty acids, vitamins, fibres, carbohydrates, proteins and minerals (Yılmaz and Bengü, 2018). Because of these nutritional contents, mushrooms are essential to consumers. Other factors such as free sugars, free amino acids, organic acids, flavour 5'-nucleotides, and volatile flavour compounds significantly affect taste and flavour. For these reasons, mushrooms have been a popular food ingredient and flavour for centuries. Today, several cultivated mushroom types can be found easily in the markets. However, wild mushrooms are preferred by consumers for their unique flavour, even if it is difficult to collect them (Xu et al., 2019).

Although it varies according to the place of growth and the type of mushroom, on average, 90% of the mushroom is water, 16-85% of dry weight is carbohydrates, 0.2-8.7% is lipids, 14-44% is proteins, 1-29% is ash. The amount of protein contained in mushrooms, which is classified as the most important protein source in developing world countries, is higher than most vegetables (Karasüleymanoğlu, 2014; Yılmaz and Bengü, 2018).

From a nutritional point of view, fresh mushrooms have high water content and neutral pH values. So microorganisms can quickly grow and thereby decrease the shelf life of fresh mushrooms to a few days. Similarly, the way of harvesting, handling and storage can affect microbiological growth. Ultimately, quality parameters like texture, colour, etc. are affected. These factors promote the growth of microorganisms, which leads to quality degradation and spoilage of fresh mushrooms (Chikthimmah and Beelman, 2006; Ergönül et al., 2018).

To our knowledge, limited scientific information is reported on the microbiological properties of wild edible mushrooms. This study aimed to assess the microbial hygienic quality of fresh wild edible mushrooms sold in Muğla province and evaluate the results in the context of public health. This data can contribute to evaluating the microbiological qualities of fresh mushrooms as highly appreciated food.

Materials and Methods

Materials

Fresh wild edible mushroom samples were used as test material in this study. Samples were purchased from ten different sellers in the Fethiye region of Muğla/Türkiye local market. All the samples were transferred to the laboratory in an icebox within the original packages. The local people don't wash these fresh mushrooms with tap water before cooking. So, the wild edible mushrooms weren't washed to represent the consumer habit in this study. The samples were analysed a few hours after purchasing, and all analyses were performed in two replications with two parallels. This study analysed

twenty samples to detect some physicochemical and microbiological properties.

Methods

Physicochemical Analyses

Mushroom samples were tested for pH and titratable acidity (anhydrous citric acid, %) to detect the physicochemical properties. These analyses were used to support the results of microbiological values.

Titratable Acidity (TA): A 10 g sample was weighed and mixed with 100 mL distilled water for homogenisation. Then, it was filtered with Whatman No. 4. 25 mL of filtered samples were taken into Erlenmeyer and phenolphthalein (Tekkim, TK.930094.00102, Türkiye) was added as an indicator. This filtrate was titrated with 0.01N sodium hydroxide (NaOH, Tekkim, TK.170511.01002, Türkiye). Accordingly, titratable acidity was calculated as anhydrous citric acid % (AOAC, 1995).

pH: pH meter (Mettler Toledo, Five easy plus FP20, China) was calibrated with two buffer solutions (pH:4.01, Mettler Toledo, 51302069, Switzerland; pH:7, Mettler Toledo, 51302047, Switzerland) before reading. Then, the pH meter detected the pH values of homogenised samples as described for titratable acidity (AOAC, 1995).

Microbiological Analyses

The microbiological analyses were designed to determine the microbiological quality of mushroom samples. A 25 g sample was weighed into the stomacher bag and then homogenised with 225 mL peptone water (0.1%) (Oxoid, LP0037, UK) by a stomacher (Isolab, 608.01.002 Germany) for 3 min. Decimal dilutions were prepared (1:10) with sterile peptone water for the enumeration of total mesophilic aerobic bacteria (TMAB), lactic acid bacteria (LAB), total yeast, total mould, *Staphylococcus aureus*, total coliform, and faecal coliform. In addition, the presence of *Escherichia coli* was investigated in all samples.

Total Count of Mesophilic Aerobic Bacteria (TMAB): Nutrient agar (NA, Biolife, 4018102, Italia) was used as a medium. The aliquots of 0.1 mL dilution were transferred into Petri dishes, and the spread plate method was performed. The Petri dishes were incubated at 30°C (Daihan Scientific, Thermo-Stable IG-105, Korea) for 24–48 h (FDA-BAM online, 2020a).

Enumeration of Yeasts and Molds: Serially diluted samples were surface plated on Potato Dextrose Agar (PDA, Biolife, 4019352, Italia). After the autoclave, PDA was aseptically acidified with 10% tartaric acid (Carlo Erba, 41127, France). The Petri dishes were incubated at 25 °C for 5 days (FDA-BAM online, 2017).

Enumeration of Lactic Acid Bacteria (LAB): The viable cell number of lactic acid bacteria was performed on de Man, Rogosa and Sharpe Agar (MRSA, Biolife, 401728S2, Italia) at 30°C for 24-48 h (ISO 15214, 1998).

Enumeration of Staphylococcus aureus: Baird Parker Agar (BPA, Biolife, 4011162, Italia) was used. After autoclave, 50% egg yolk (Merck, 1.03784.0001, UK) was added into the cooling medium at 50 °C. 3.5% tellurite (Aldrich, P0677-25G, Japan) was sterilised by a membrane filter and put in the medium. The Petri dishes were incubated at 37 °C for 24-48 hours (FDA-BAM online, 2019).

Enumeration of Total Coliform and Faecal Coliform, Detection of E. coli: Total coliform and faecal coliform were detected by the most probable number (MPN) technique. 1 mL of aliquots of serial dilution were inoculated into three tubes containing Lauryl Sulphate Tryptose Broth (LSTB, Merck, 1.10266.0500, Germany). LSTB tubes were incubated at 37 °C for 24–48 h. After incubation of gas positive tubes were determined. A loopful of suspension from each gassing LSTB tube was transferred to Brilliant Green Bile Broth (BGLB, Merck, 1.05454.0500, Germany) and *Escherichia coli* Broth (ECB, Biolife, 4014252, Italia). BGBB and ECB were incubated at 37 °C for 24–48 h and 45 °C for 24–48 h, respectively. Then, BGBB and ECB tubes were controlled for gas production. Confirmed coliform and faecal coliform were indicated in BGBB and ECB medium. The detection of *E. coli* was examined on Eosin Methylene Blue Agar (EMBA, Merck, 1.01347.0500, Germany) from the gas-positive ECB tubes. Typical colonies were confirmed by IMViC tests (FDA-BAM online, 2020b).

Statistical Analyses

All experiments had two replicates and two parallels (n=4). All statistical analyses were performed with the SPSS statistical package program (IBM SPSS Statistics Version 22; USA) by ANOVA variance analysis. The significance levels of $P < 0.05$ were used for statistical differences. Duncan Tests established the significant difference between the means.

Results and Discussion

Physico-chemical properties such as titratable acidity and pH were determined to evaluate the results of microbiological tests, as shown in Table 1. The results of titratable acidity analyses ranged from 0.005-0.020% (anhydrous citric acid %). The highest titratable acidity was shown in sample 9.

Sample 9 was significantly different (P<0.05). The samples' lowest and highest pH values were 6.82 and 7.54. The pH values of the mushroom were similar (P>0.05). The pH values correlated with the titratable acidity values. The food has been classified depending on the pH value. Low-acid foods have a pH greater than 5.7, meaning they contain low amounts of acid (Temiz, 1999). The average value of the pH was 7.18 for mushroom samples. So, the mushroom samples can be considered as low-acid foods.

Table 1. Physico-chemical properties of wild edible mushroom*

Sample	pH	Titratable acidity (TA) (anhydrous citric acid %)
1	7.08 ±0.01^{abcde}	0.008 ±0.001^{ab}
2	6.96 ±0.28^{ab}	0.006 ±0.003^{a}
3	6.97 ±0.20^{abc}	0.009 ±0.000^{ab}
4	7.28 ±0.06^{defgh}	0.009 ±0.000^{ab}
5	7.33 ±0.01^{efgh}	0.009 ±0.001^{ab}
6	7.23 ±0.08^{cdefg}	0.008 ±0.002^{ab}
7	7.34 ±0.01^{efgh}	0.005 ±0.001^{a}
8	7.39±0.13^{fgh}	0.007 ±0.001^{a}
9	6.82 ±0.24^{a}	0.020 ±0.006^{d}
10	7.19 ±0.11^{bcdefg}	0.009 ±0.002^{ab}
11	7.02 ±0.03^{abc}	0.007 ±0.001^{a}
12	7.15 ±0.00^{bcdef}	0.008 ±0.000^{ab}
13	7.01 ±0.04^{abc}	0.012 ±0.001^{cb}
14	6.97 ±0.02^{abc}	0.013 ±0.001^{c}
15	7.54 ±0.01^{h}	0.005 ±0.001^{a}
16	7.12 ±0.10^{bcde}	0.008 ±0.000^{ab}
17	7.44 ±0.04^{gh}	0.005 ±0.000^{a}
18	7.44 ±0.08^{gh}	0.006 ±0.000^{a}
19	7.11 ±0.01^{bcde}	0.014 ±0.001^{c}
20	7.19 ±0.09^{bcdefg}	0.008 ±0.001^{ab}

**n=4, (± standard deviation);* Significant difference was detected between the values in the column (p<0.05).

It has been established that most microorganisms grow best at pH values around 7.0 (6.6-7.5) (Jay, 2000). The results of titratable acidity and pH of the samples are very favourable for the growth of numerous microbial species can be expected (Roberts et al., 2005). The analyses of TMAB, LAB, total yeast, total mould, *S. aureus*, total coliform, faecal coliform, and *E. coli* were performed to determine the initial microflora of mushroom samples.

Ordinary healthy mushrooms have high bacterial populations. The results of enumeration of TMAB ranged between 4.13 and 8.83 log CFU/g (Table 2). Similarly, total bacterial numbers ranged from 6.3 to 7.2 log CFU/g of fresh mushroom tissue, as demonstrated by Chikthimmah and Beelman, 2006. According to Venturini et al. (2011), TMAB counts of wild edible mushrooms from Spain were between 4.4 log CFU/g and 9.4 log CFU/g. TMAB values were presented between 6.8 and 9.2 log CFU/g for eight wild edible mushrooms from Türkiye (Ergönül et al., 2018). Three cultivated mushroom species were analysed, and their mesophilic aerobic counts were expressed as 4.87, 5.00, and 7.92 log CFU/g (Reyes et al., 2004).

LAB was present in relatively low numbers compared to the TMAB, with <1.00- 5.57 log CFU/g range results. The highest enumeration of LAB was determined in sample 3, and the lowest one was in sample 2 and sample 12 (Table 2). LAB counts of mushroom samples were investigated between 1.4 and 2.4 log CFU/g (Ergönül et al., 2018). The mean value of LAB was reported as 2.1 log CFU/g by Venturini et al. (2011). The average result of this study was higher. It came out as 3.70 log CFU/g. In another study, *Lactobacillus* sp. and *Pediococcus* sp. were isolated from fresh mushrooms. However, the enumeration was not declared (Halami et al., 1999).

S. aureus produces a staphylococcal enterotoxin that is extremely thermotolerant, which means it can survive the cooking process (Grispoldi et al., 2021). Detectable enumeration of *S. aureus* cells was not observed in most mushroom samples except sample 11 (Table 2). No significant differences (P>0.05) were detected in the samples (except sample 11). Venturini et al. (2011) and Ergönül et al. (2018) stated that S. aureus was not isolated in any of the samples examined.

Mushrooms also contain significant levels of yeasts and moulds. The count of yeast was established to be between 3.10 log CFU/g and 7.76 log CFU/g. The mould values of fresh mushrooms were determined as <1.00 and 3.93 log CFU/g (Table 2). Chikthimmah and Beelman (2006) showed that freshly harvested mushroom samples had approximately 3 log CFU of moulds and 6 log CFU of yeast per gram. Although the yeast population increased to 6.9 log CFU/g, mould counts were stable after 6 days of storage at 4°C. The total count of yeast and mould samples was higher than the previous study of Ergönül et al. (2018), who determined it to be between 1.9 and 3.3 log CFU/g. The yeast and mould results of mushroom samples determined by Venturini et al. (2011) were also lower than those indicated in the present study.

Table 2. Microbiological properties of wild edible mushroom

Sample	TMAB log CFU/g	LAB log CFU/g	*S. aureus* log CFU/g	Total Count of Yeast log CFU/g	Total Count of Mold log CFU/g	Total Coliform log MPN/g	Faecal Coliform log MPN/g
1	8.55 ±0.17^{ijk}	3.31 ±0.05^{b}	<1.00 ±0.00^{a}	5.17 ±0.19^{fg}	3.93 ±0.13^{h}	3.90 ±0.39^{f}	3.90 ±0.39^{c}
2	7.50 ±0.09^{bcd}	<1.00 ±0.00^{a}	<1.00 ±0.00^{a}	3.40 ±0.22^{ab}	3.52 ±0.11^{efg}	<0.30 ±0.00^{a}	<0.30 ±0.00^{a}
3	6.10 ±0.06^{a}	5.57 ±0.11^{ı}	<1.00 ±0.00^{a}	3.10 ±0.14^{a}	3.18 ±0.10^{bc}	2.80 ±0.24^{d}	<0.30 ±0.00^{a}
4	7.96 ±0.04^{efg}	4.67 ±0.22^{efg}	<1.00 ±0.00^{a}	4.60 ±0.24^{def}	3.36 ±0.12^{cdef}	2.20 ±0.23^{bc}	1.76 ±0.29^{b}
5	8.01 ±0.24^{efg}	4.44 ±0.15^{ef}	<1.00 ±0.00^{a}	3.61 ±0.42^{abc}	3.15 ±0.14^{bc}	<0.30 ±0.00^{a}	<0.30 ±0.00^{a}
6	7.22 ±0.27^{b}	5.22 ±0.05^{h}	<1.00 ±0.00^{a}	4.02 ±0.60^{bcd}	<1.00 ±0.00^{a}	3.07 ±0.15^{de}	1.56 ±0.00^{b}
7	7.29 ±0.28^{bc}	3.49 ±0.07^{b}	<1.00 ±0.00^{a}	3.40 ±0.17^{ab}	3.29 ±0.14^{cde}	<0.30 ±0.00^{a}	<0.30 ±0.00^{a}
8	8.83 ±0.32^{k}	4.75 ±0.24^{fg}	<1.00 ±0.00^{a}	5.45 ±0.04^{g}	3.23 ±0.11^{bc}	1.96 ±0.00^{bd}	1.76 ±0.29^{b}
9	8.15 ±0.16^{fgh}	3.91 ±0.11^{c}	<1.00 ±0.00^{a}	4.75 ±0.18^{ef}	3.73 ±0.10^{hg}	2.80 ±0.24^{d}	<0.30 ±0.00^{a}
10	7.89 ±0.13^{ef}	<1.00 ±0.00^{a}	<1.00 ±0.00^{a}	7.23 ±0.17^{h}	3.33 ±0.17^{cde}	2.20 ±0.23^{bc}	1.76 ±0.29^{b}
11	8.31 ±0.02^{ghı}	4.97 ±0.43^{gh}	4.74 ±0.35^{b}	4.57 ±0.38^{def}	3.60 ±0.15^{fg}	5.07 ±1.26^{g}	5.07 ±1.26^{d}
12	8.78 ±0.01^{jk}	<1.00 ±0.00^{a}	<1.00 ±0.00^{a}	3.52 ±0.19^{abc}	3.35 ±0.08^{cdef}	<0.30 ±0.00^{a}	<0.30 ±0.00^{a}
13	7.38 ±0.01^{bcd}	3.41 ±0.09^{b}	<1.00 ±0.00^{a}	3.12 ±0.06^{a}	3.02 ±0.08^{b}	2.20 ±0.23^{bc}	<0.30 ±0.00^{a}
14	8.30 ±0.13^{ghı}	4.43 ±0.13^{ef}	<1.00 ±0.00^{a}	4.19 ±0.41^{cde}	3.22 ±0.06^{bc}	2.20 ±0.23^{bc}	1.76 ±0.29^{b}
15	7.69 ±0.15^{de}	4.10 ±0.09^{cd}	<1.00 ±0.00^{a}	4.10 ±0.53^{cde}	<1.00 ±0.00^{a}	3.07 ±0.15^{de}	<0.30 ±0.00^{a}
16	4.13 ±0.22^{cde}	4.73 ±0.05^{efg}	<1.00 ±0.00^{a}	4.54 ±0.41^{def}	3.26 ±0.15^{bcd}	2.50 ±0.19^{bcd}	<0.30 ±0.00^{a}
17	7.37 ±0.20^{bcd}	4.07 ±0.11^{c}	<1.00 ±0.00^{a}	4.32 ±0.21^{de}	3.34 ±0.02^{cde}	2.20 ±0.23^{bc}	1.56 ±0.00^{b}
18	8.07 ±0.07^{fgh}	4.48 ±0.04^{ef}	<1.00 ±0.00^{a}	5.58 ±0.08^{g}	3.47 ±0.07^{def}	3.07 ±0.15^{de}	2.00 ±0.06^{b}
19	8.25 ±0.09^{fghı}	4.39 ±0.08^{de}	<1.00 ±0.00^{a}	4.36 ±0.21^{de}	3.53 ±0.05^{efg}	3.80 ±0.24^{ef}	<0.30 ±0.00^{a}
20	8.43 ±0.09^{hıj}	4.10 ±0.07^{cd}	<1.00 ±0.00^{a}	7.76 ±0.34^{h}	<1.00 ±0.00^{a}	2.00 ±0.06^{bc}	<0.30 ±0.00^{a}

n=4, (± standard deviation); Significant difference was detected between the values in the column ($p<0.05$).

Since coliform bacteria are common in both the intestines and nature (soil, plants, etc.), they are considered indicators of sanitation in the food industry. High levels of coliform microorganisms in food indicate that the required hygienic measures have not been taken during or after harvesting, storage, and sale (Frazier and Westhoff, 1988; Jay, 2000). Total coliform counts of the samples were obtained between 0.30 and 5.07 log MPN/g (Table 2). 80% of the mushroom samples were harbouring total coliform. "Faecal coliform" within the coliform group, located in the natural flora of the intestine system of humans and warm-blooded animals, is considered an indicator of faecal contamination. It is known that most bacteria identified as faecal coliforms in the coliform group are *E. coli*. The presence of *E. coli* and/or faecal coliform bacteria in any sample implies that the sample is directly or indirectly contaminated with faeces and may contain other intestinal pathogens. For this reason, *E. coli* and faecal coliforms are not allowed in any foodstuff, drinking, and/or utility water (Khan and Gupta, 2020; Schalli et al., 2022). The faecal coliform was found in 45% of the tested samples. Faecal coliforms were not detected in samples 2, 3, 5, 7, 9, 12, 13, 15, 16, 19 and 20 (Table 2). The highest results were in sample 11 for both total and faecal coliform. Similarly, eight wild edible mushrooms were tested for total coliform and mentioned 0.3-1.4 log CFU/g in four samples (50%) (Ergönül et al., 2018). In another study, coliform bacteria were detected in 23.4% of the tested mushroom samples (Venturini et al., 2011). Based on confirmation and identification tests, *E. coli* Biotype I was found in 35% of the tested samples (1, 4, 6, 8, 11, 17, and 18) and *Enterobacter aerogenes* in only one sample (sample 10). The environment of mushrooms, especially those grown in natural ecosystems, makes them vulnerable to pathogens since this can be excreted in the faeces of animals and insects and transmitted to the ascocarps.

Conclusion

Unlike fruits and vegetables (whole or minimally processed), information on the microbial quality of fresh mushrooms is minimal, especially for commercial wild mushroom samples. As in other studies, the fresh mushroom species tested in this study were characterised by a high microbial load. Fresh mushrooms favour growing microorganisms due to their high

moisture character with carbohydrates, protein, vitamins, and minerals. From the standpoint of nutrients, fresh mushrooms are capable of causing quality degradation and spoilage.

Soil, water, air, insects, and animals impact the microbial flora of fresh mushrooms. The high count of bacteria diminishes microbial quality. The initial microbial load can affect the deterioration during postharvest. Picked fresh wild edible mushrooms may be placed into bags, boxes, or crates without washing. When harvesting, personal hygiene must be practised by pickers to prevent the transfer of human pathogens. Notably, pathogens such as faecal coliform and E. coli in mushrooms indicate that proper hygiene and sanitation rules weren't followed during picking, transportation, retailing and storage. Mushrooms can be contaminated with these microorganisms in nature, too. During storage, the fingers and shoes of people handling the mushrooms, their baskets, and knives can contaminate them. So, in general, the microbiological quality of the mushrooms sold in Muğla province is poor and threatens human health. The determination of yeast and mould, the presence of mycotoxin, and other existence of enteropathogens will be investigated in further studies.

Compliance with Ethical Standards

Conflict of interests: The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study includes no experiments with human or animal subjects.

Data availability: Data will be made available on request.

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